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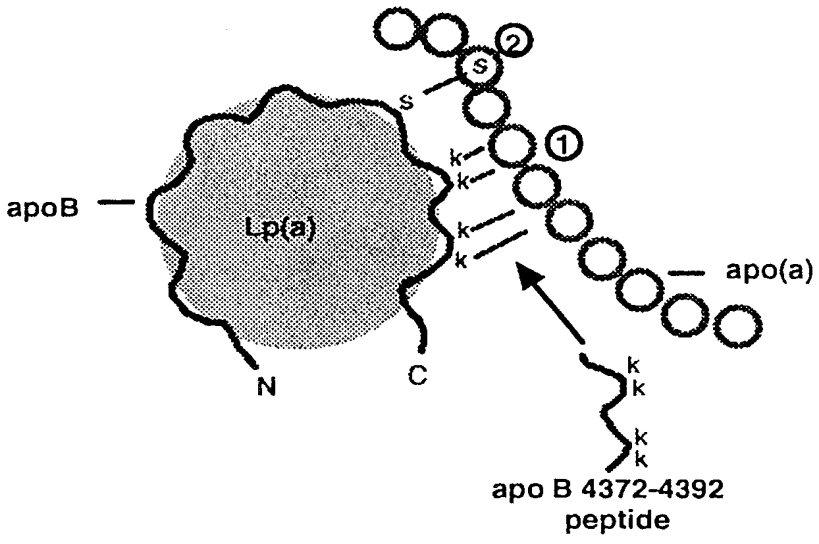
(54) Title: INHIBITION OF LIPOPROTEIN FORMATION

(57) Abstract

Proteins which are effective inhibitors of lipoprotein(a) formation are provided, generally as peptides which correspond to region apoB 4372 to 4392 or as antibodies which bind that region. Medicaments which comprise such peptides or antibodies are also provided. The proteins and medicaments are useful in therapy, including in the treatment of atherosclerosis.



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INHIBITION OF LIPOPROTEIN FORMATION

This invention relates to inhibition of lipoprotein(a) formation. In particular, it relates to peptides and antibodies which have the capability of at least partially inhibiting the formation of lipoprotein(a).

BACKGROUND

Lipoprotein(a) is a cholesterol-rich lipoprotein formed in human plasma by the linkage of apolipoprotein (apo) B, on a low density lipoprotein (LDL) particle, to apo(a) (Utermann (1989), McLean *et al* (1987)). Elevated levels of lipoprotein(a) have recently been identified as an independent risk factor for developing atherosclerosis. The atherogenic nature of lipoprotein(a) is supported by its presence in human atherosclerotic tissue (Rath *et al* (1989), Cushing *et al* (1989)) and by many human studies that show a positive link between high plasma lipoprotein(a) levels and the risk of developing heart disease (Dahlen *et al* (1986), Kostner *et al* (1981), Berg *et al* (1997)).

Existing lipid-lowering drugs have not proven successful at lowering plasma lipoprotein(a) levels (Berg *et al* (1989), Hajjar *et al* (1996), Hunninghake *et al* (1993)). The general object of this invention is therefore to provide a molecule that will at least partially inhibit lipoprotein(a) formation and hence have the potential to be used as a lipoprotein(a)-lowering agent.

Most evidence suggests that lipoprotein(a) is formed in circulation after independent secretion of LDL and apo(a) from the liver (Chiesa *et al* (1992), White (1995)). It is generally accepted that the linkage of apoB to apo(a) is a two-step process (Trieu *et al* (1995), Brunner *et al* (1993)). The first step is an initial noncovalent binding of apoB to apo(a), while the second step is the formation of a disulphide bridge between apo(a)Cys4057 and apoBCys4326. While the second step of lipoprotein(a) assembly has been well characterised (Brunner *et al* (1993), McCormick *et al* (1995)), the protein sequences involved in the initial noncovalent binding step have not been well defined. Lysine residues have been implicated in this first-step, since lysine analogues disrupt lipoprotein(a) formation (Chiesa *et al* (1992)) and the apo(a) protein is known to bind lysine (Eaton *et al* (1987), Guevara *et al* (1993)).

Previous work in relation to transgenic mice expressing truncated apoB mutants has identified a general region in the carboxyl-terminus of apoB (amino acids 4331-4397) that, if removed by truncation, severely impaired lipoprotein(a) formation (McCormick *et al* (1997)). However, the work undertaken to date has not identified which amino acids within this general region are responsible for non-covalent binding of apo(a).

The applicants have now identified a specific region of apoB which non-covalently binds apo(a). It is this finding by the applicants which has led to the present invention.

SUMMARY OF THE INVENTION

Accordingly, in a first aspect, the invention provides a peptide which at least partially inhibits lipoprotein(a) formation which comprises the following amino acid sequence:

R_1 X X X X X X R_2 X X X X X X R_3 X X X X X X R_4

wherein R_1, R_2, R_3 and R_4 are each independently selected from lysine, arginine and histidine, and wherein each X is an amino acid other than lysine, arginine or histidine.

Preferably, R_1, R_2, R_3 and R_4 are each lysine.

Alternatively, R_1, R_2, R_3 and R_4 are each arginine.

In another embodiment, the invention provides a peptide which at least partially inhibits lipoprotein(a) formation which comprises the following amino acid sequence:

K Y Y E L E E K I V S L I K N L L V A L K

or a functional equivalent thereof.

In a further embodiment, the invention provides a peptide which has an alpha-helical structure and which comprises the following amino acid sequence:

K X X X X X X K X X X X X X K X X X X X X K

wherein X is any amino acid other than lysine, and wherein said peptide is capable of at least partially inhibiting lipoprotein(a) formation.

In still a further aspect, the invention provides a peptide which is at least 21 amino acid residues in length, which forms an alpha-helical structure with four surface residues independently selected from lysine, arginine or histidine and which is capable of at least partially inhibiting lipoprotein(a) formation.

Preferably, the peptide includes four surface lysine residues.

Alternatively, the peptide includes four surface arginine residues.

In another aspect, the invention provides a polynucleotide which encodes a peptide as defined above.

In yet another embodiment, the invention provides antibodies which bind to a peptide as defined above and which at least partially inhibit lipoprotein(a) formation.

In still another embodiment, the invention provides antibodies which bind to the region spanning amino acids 4372 to 4392 of apoB.

In still a further embodiment, the invention provides an anti-idiotypic antibody which mimics the conformation of the region spanning amino acids 4372 to 4392 of apoB.

In still a further aspect, the invention provides a medicament which comprises a peptide or antibody as defined above in a pharmaceutically acceptable form, said peptide or antibody being present in an amount sufficient to inhibit lipoprotein(a) formation.

In still a further aspect, the invention provides for the use of a peptide or antibody as defined above in the manufacture of a lipoprotein(a) lowering medicament.

In yet a further aspect, the invention provides a method of lowering lipoprotein(a) levels in plasma in a patient comprising the step of administering to said patient an amount of a peptide, antibody or medicament as defined above which is effective to at least partially inhibit new lipoprotein(a) formation.

In a final aspect, the invention provides a method of lowering lipoprotein(a) levels in a patient comprising the step of preventing or reducing the non-covalent binding of apoB region apoB 4372-4392 to apo(a).

DESCRIPTION OF THE DRAWINGS

Although the invention is broadly as defined above, it will be appreciated by those persons skilled in the art that it also includes embodiments of which the following description provides examples. In addition, the invention will be better understood by reference to the accompanying drawings in which:

Figure 1 shows a strategy to disrupt lipoprotein(a) formation using a synthetic apoB peptide. The two step model of lipoprotein(a) assembly is shown. In the first step, surface lysine residues in the apoB4372-4392 region bind to lysine-binding sites on the apo(a) molecule bringing the apoBCys4326 and apo(a)Cys4057 residues in close proximity. Lipoprotein(a) assembly is completed in the second step with the formation of a disulphide bond between the two cysteines. The strategy followed in the present invention was to introduce a synthetic apoB peptide spanning apoB4372-4392 to compete with the apoB molecule for binding to apo(a) and therefore inhibit the first step of lipoprotein(a) assembly.

Figure 2 shows the predicted alpha helix formed by the apoB602 peptide. Computer analysis of the apoB602 sequence using the HELICALWHEEL programme predicts an amphipathic alpha helix. Hydrophobic resides (shown boxed) are predicted to be buried in the lipid phase while the hydrophilic resides project into the aqueous phase. Two sets of paired lysine residues (K) are located on opposite sides of the lipid/aqueous interface.

Figure 3 shows the Western blots of lipoprotein(a) formation in incubations containing apoB synthetic peptides. Aliquots (20μl) from 3-h incubations containing human apo(a) and apoB and increasing amounts of either the apoB602, arg602 or scram602 peptide, were subject to SDS-PAGE on 4% polyacrylamide gels under non-reducing conditions. Western blot analysis was performed with the human apo(a)-specific monoclonal antibody, al-1, which recognises both lipoprotein(a) and free apo(a).

Figure 4 shows inhibition of lipoprotein(a) formation by apoB peptides. The ability of the apoB peptides to inhibit lipoprotein(a) formation *in vitro* was quantified using a sandwich enzyme-linked immunoassay (ELISA). Increasing amounts of peptide (from 0 to 200μM) were added to incubations containing 1μl of apo(a) and 1μl of apoB transgenic mouse plasma. The amount of lipoprotein(a) formed in each incubation was quantified in triplicate with a sandwich ELISA. Sigmoidal curves were fitted to all data points for each peptide in the Microsoft Excel programme and IC50 values were derived for each peptide.

Figure 5 shows the half-life of peptide apoB602 in mice. The half-life of radiolabelled apoB602 was calculated in wildtype and transgenic mice. ^{125}I-radiolabelled peptide was injected into mice and an initial total cpm was calculated from time zero plasma samples. Blood samples were then taken at set time points and the percent of the initial total cpm was calculated for each time point. These percentages were graphed on a $\log_{10}$ scale against time. The decay lines were used to calculate the half-lives in wildtype and transgenic mice.

Figure 6 shows Western blots of plasma from lipoprotein(a) mice after administration of the arg602 peptide. Pairs of transgenic mice containing lipoprotein(a) in their plasma were administered either 200 μl of saline via tail vein injection, or 200 μl of a 10 mg/ml solution of the arg602 peptide in saline (total dose = 2mg), Blood samples were taken at 0, 0.17, 0.5 1, 2, 4, and 8 hrs after injection and the levels of lipoprotein(a) versus fee apo(a) were visualised by western blot analysis.

DESCRIPTION OF THE INVENTION

As broadly defined above, the primary focus of the invention is on proteins which are capable of at least partially inhibiting the formation of lipoprotein(a). This inhibitory function makes the proteins suitable for use in the lowering of lipoprotein(a) levels in plasma.

In one form, the proteins are peptides. The peptides of the invention are generally at least 21 amino acid residues in length. They may have a number of amino acid sequences.

One such amino acid sequence is as follows:

R_1 X X X X X X R_2 X X X X X X R_3 X X X X X X R_4

wherein R_1, R_2, R_3 and R_4 are each independently selected from lysine, arginine and histidine, and each X is an amino acid other than lysine, arginine or histidine.

Another amino acid sequence is as follows:

K X X X X X X K X X X X X X K X X X X X X K

In this sequence, each X can be any amino acid other than lysine, more preferably any amino acid other than lysine, arginine or histidine.

Still another such amino acid sequence is:

K Y Y E L E E K I V S L I K N L L V A L K

The peptide of this invention is believed to form an alpha-helical structure, usually with four lysine residues as paired surface residues. One or more of the lysine residues can however be replaced by arginine or histidine residue(s).

The present invention also contemplates functional equivalents of the specific peptide sequences above. Such functional equivalents are those in which individual

amino acid residues from within the specific sequence are replaced by other individual amino acid residues without substantially affecting the functionality of the resulting peptide as an inhibitor of lipoprotein(a) formation.

In this regard, the following amino acids are often considered as replacing one for the other without substantially affecting functionality:

(a) A, S, T, P and G;
(b) N, D E and Q;
(c) H, R and K;
(d) M, L, I and V; and
(e) F, Y and W.

As mentioned above, the important lysine residues can be replaced by arginine residues. For example, a peptide in which all four lysine residues are replaced to give four arginine surface residues is a preferred (and functionally equivalent) variant. This peptide has the sequence:

R Y Y E L E E R I V S L I R N L L V A L R

As indicated, the peptide of the invention will not generally have less than 21 amino acid residues but can have more. Longer sequences (containing, for example, from 22 to 40 amino acids) which form stable alpha-helical structures are expressly contemplated.

An example of such a sequence is the following 32 amino acid peptide:

S I V G W T V K Y Y E L E E K I V S L I K N L L V A L K D F H S.

The peptides can also be provided as dimers or trimers of smaller peptides, such as dimers or trimers of the 21 amino acid peptide above.

The peptides can be prepared using any conventional approach. Such methods include protein synthesis from individual amino acids as described by Stuart and Young in "Solid Phase Peptide Synthesis", Second Edition, Pierce Chemical

Company (1984). This is the presently preferred preparative route given the short length of the peptides, although it is by no means intended that other synthetic routes (including recombinant expression if appropriate) are excluded. Should that be required, standard techniques can be employed as are generally described by Sambrook *et al* (1987).

For use in recombinant techniques, polynucleotides which encode the peptides are provided. The precise nucleotide sequence of the polynucleotides will vary depending upon the amino acid sequence of the peptide to be expressed as well as the degeneracy of the nucleic acid code. However, an exemplary polynucleotide according to the invention is the following:

AAA TAT TAT GAA CTT GAA GAA AAG ATA GTC AGT CTG ATC AAG AAC

CTG TTA GTT GCT CTT AAG

Once obtained, the peptides of the invention can be formulated into medicaments. Such medicaments can include solid dosage forms or liquid dosage forms, whichever is appropriate. Dependent upon the formulation selected and the route of administration, the medicament will contain pharmaceutically acceptable carriers, excipients, and be prepared by any conventional approach.

Injectable formulations are presently preferred, although many other formulations which provide for delivery of the peptides in an active form (such as oral formulations including microencapsulated peptides and transdermal patches and the like) are also applicable.

The dosage of peptide employed will be dependent upon the peptide and the selected route of administration. Determination of a specific dosage will be routine to the art-skilled worker in this field.

Antibodies to the apoB 4372-4392 region are also proteins provided by this invention. Such antibodies can be polyclonal but will preferably be monoclonal antibodies. Monoclonal antibodies with affinities of 10^{-8} M^{-1} or preferably 10^{-9} to 10^{-10} M^{-1} or stronger will typically be made by standard procedures as described, eg. in Harlow & Lane (1988). Briefly, appropriate animals will be selected and the desired

immunization protocol followed. After the appropriate period of time, the spleens of such animals are excised and individual spleen cells fused, typically, to immortalised myeloma cells under appropriate selection conditions. Thereafter, the cells are clonally separated and the supernatants of each clone tested for their production of an appropriate antibody specific for the desired region.

Other suitable techniques for preparing antibodies involve *in vitro* exposure of lymphocytes to the antigenic polypeptides, or alternatively, to selection of libraries of antibodies in phage or similar vectors. See Huse *et al.,* 1989.

Also, recombinant immunoglobulins may be produced using procedures known in the art (see, for example, US Patent 4,816,567 and Hodgson (1991)).

Anti-idiotypic antibodies raised against antibodies to the apoB 4372-4392 region are also contemplated. Such anti-idiotypic antibodies will mimic the conformation of the region. These antibodies have applications equivalent to the peptides discussed above in inhibiting lipoprotein(a) formation.

The invention will now be described with reference to the following experimental section which will be understood to be exemplary only and not to function as a limitation of the scope of protection sought.

EXPERIMENTAL

MATERIALS

ApoB peptides

The sequences of the four apoB peptides used in this experiment are listed in Table 1.

Table 1. Characteristics of apoB synthetic peptides

Peptide	Sequence	No of residues	M. Wt.	Purity
APOB602	KYYELEEKIVSLIKNLLVALK	21	2507	>78%
APOB602L	SIVGWTVKYYELEEKIVSLIKNLLVALKDFHS	32	3737	>77%
SCRAM602	VKEKIYSKLILKNLEVAYELL	21	2507	>95%
ARG602	RYYELEERIVSLIRNLLVALR	21	2619	>91%

The apoB602 peptide corresponds to amino acids 4372-4392 in the apoB carboxyl-terminus. Arg 602 spans the same sequence, however includes replacement of all four lysines in the apoB602 sequence with arginine residues. The Scram602 peptide is a scrambled version of the apoB602 sequence and apoB602L peptide is a longer version of the apoB602 peptide spanning the entire predicted alpha helix in this region (Segrest *et al* (1998). All four apoB peptides were chemically synthesised using the solid phase method (Valerio *et al* (1994)) by Chiron Technologies (Clayton, Australia). The peptides were lyophilised and stored in the dark under vacuum until use. To prepare the peptides for the lipoprotein(a) formation assays all peptides were either dissolved in 0.1% acetic acid or sterile saline.

Recombinant human apo(a) and apoB

The human apo(a) used for the *in vitro* lipoprotein(a) formation assays was obtained from the plasma of transgenic mice expressing human apo(a) (Chiesa *et al* (1992)). The human apoB was obtained from the plasma of transgenic mice expressing human apoB100 (Linton *et al* (1993)).

EXPERIMENT 1

Lipoprotein(a) formation assays

All peptides were tested for their effect on lipoprotein(a) formation in a standard Western blot-based lipoprotein(a) formation assay (McCormick *et al* (1994)). An increasing amount of peptide (from 0 to 280μM) was added to incubations containing human apo(a) from transgenic mouse plasma (1.0 μL) and human apoB100 from transgenic mouse plasma (2.0 μL, equivalent to approximately 1μg of apoB). Incubations were performed in duplicate in 0.15M NaCl in a total volume of 40μL for 3 hours at 37°C. The incubations were subjected to electrophoresis on

SDS-4% polyacrylamide gels under non-reducing conditions and the separated proteins transferred to nitrocellulose. Western blot analysis was performed with the human apo(a)-specific monoclonal antibody a1-1 (Marcovina *et al* (1995)) conjugated to horse-radish peroxidase. The amount of lipoprotein(a) formed in each incubation was visualised after detection with Enhanced Chemiluminesence reagents (Amersham Corp.).

The ability of the apoB peptides to inhibit lipoprotein(a) formation *in vitro* was quantified using an sandwich enzyme-linked immunoassay (ELISA) performed in 96 well ELISA plates. Increasing amounts of peptide (from 0 to 200μM) were added to incubations containing 1μl of apo(a) and 1μl of apoB transgenic mouse plasma. In separate incubations, increasing amounts of a lysine analogue, ε-amino caproic acid (0-100mM) were added. Incubations were performed in PBS containing 2% BSA for 3 hours at 37°C. The amount of lipoprotein(a) formed in each incubation was quantified in triplicate with an sandwich ELISA which uses an apo(a)-specific 'capture' and an apoB-specific 'detection' monoclonal antibody. Plates were developed after incubation with an HRP-labelled anti-mouse IgG Antibody. Controls to quantify background binding included incubations containing apo(a) or apoB only, as well as apo(a)/apoB incubations containing 100 nM ε-amino caproic acid which completely inhibits lipoprotein(a) formation (Chiesa *et al* (1992)). Sigmoidal curves were fitted to all data points for each peptide in the Microsoft Excel programme and IC50 values were derived for each peptide.

RESULTS

Peptide structure

Computer analysis of the apoB602 sequence on which the tested peptides are based using the HELICALWHEEL programme predicts that this sequence would form an alpha helix containing two sets of paired lysine residues at opposite sides of the aqueous/water interface (Figure 2). Analysis of the scram602 control peptide predicted that the scrambled sequence would disrupt both the alpha helical structure and placement of the lysine residues.

Lipoprotein(a) formation assays

To test the peptides for their ability to inhibit lipoprotein(a) formation *in vitro,* the peptides were placed into a standard lipoprotein(a) formation assay and measured

by two separate methods; a Western-blot based method which visualises the amount of lipoprotein(a) and free apo(a) in the incubations; and a sandwich ELISA which quantifies the amount of lipoprotein(a) formed in each incubation. Results from Western blot analysis (Figure 3) showed the arg 602 peptide to be the most effective inhibitor of lipoprotein(a) formation *in vitro,* showing almost complete inhibition of lipoprotein(a) formation at around 33μM. This was in keeping with data gained from the ELISA assay (Figure 4) which showed a IC50 value for the arg602 peptide of 20μM. The apoB602 peptide also showed inhibition of lipoprotein(a) formation in the μM range with a IC50 value of øf 78μM. A longer version of the apoB602 peptide (apoB602L) was less effective as an inhibitor of lipoprotein(a) formation with a IC50 value of 375μM. In contrast, the scram602 peptide showed no effect on lipoprotein(a) formation over the range of concentrations tested in the Western blot assay although some inhibition was seen in the ELISA assay at the highest concentration (200 μM). Interestingly, all peptides tested had IC 50 values well below that of ε–amino caproic acid (IC50 = 12 mM, inset, Figure 4) a lysine analogue commonly used as an *in vitro* inhibitor of lipoprotein(a) formation (Frank *et al* (1995)).

As expected, inhibition of lipoprotein(a) formation by the peptides resulted in a corresponding increase in the amount of free apo(a) in the incubations as visualised in the Western blot assays (Figure 3).

DISCUSSION

The focus of the above work is a peptide which comprises a highly conserved stretch of 21 amino acids (apoB4372-4392). It is believed that the peptide mimics the natural apo(a) binding site on the apoB molecule and competes with native apoB for binding to apo(a).

The ability of the peptide (apoB602) to inhibit lipoprotein(a) formation was tested in a standard lipoprotein(a) formation assay. The results indicate that the apoB602 peptide is an effective inhibitor of lipoprotein(a) formation. Lipoprotein(a) formation was almost completely inhibited in incubations containing 70 μm of the apoB602 peptide. A control peptide (scram602) containing the same sequence, only scrambled, had no effect on lipoprotein(a) formation.

Structural analysis of the apoB602 peptide predicts that the sequence forms an alpha helix. This region of apoB is contained within apoB sequences previously found to form a class A alpha helix and constituting an important lipid-binding site (Segrest *et al* (1998)). A striking feature of the alpha helix formed by the apo4372-4392 sequence is the presence of paired lysine residues on opposite sides of the interface between the lipid and aqueous phases. Lysine residues have been implicated in the first step of lipoprotein(a) assembly since lysine analogues can block the formation of lipoprotein(a) *in vitro*. The results obtained suggest that the alpha helix containing paired surface lysines forms an important binding motif that binds to the lysine binding sites in apo(a). The alpha helix and presence of lysine residues in this putative apo(a) binding site are both important structural features. The scram 602 peptide sequence disrupts both features and renders the peptide inactive as an lipoprotein(a) inhibitor.

To evaluate whether the interaction of the apoB602 peptide was entirely dependent on the four lysine residues or whether it is more dependent on positive charge, a further peptide (arg602) in which the four lysine residues were replaced with arginines was tested for its effect on lipoprotein(a) formation.

The arg602 peptide proved to be an even more effective inhibitor than the apoB602 peptide in both the Western blot and ELISA assays. These results show clearly that replacement of one positively charged amino acid residue (lysine) with another (arginine) can be effected without substantially affecting functionality of the peptide. The alpha helical structure is however expected to be retained in the arg602 peptide.

EXPERIMENT 2

***In vivo* testing of the apoB peptides.**

***In vivo* half life**

The *in vivo* stability of peptide 602 was measured in both wildtype and apo(a) transgenic mice. Two C57/Bl6 male wild-type mice and two apo(a) transgenic male mice were injected via the tail vein with ^{125}I-radiolabelled peptide (5 x 10^6 counts per

mouse) diluted in 0.15M sterile NaCl. Blood samples were collected from the mice at times 0, 10 min, 30 min, 1 hr, 2 hrs, 4 hrs, 8hrs and 24hrs. Aliquots (40µl) of blood taken at each time point, were centrifuged, and the plasma was measured for radioactivity in a gamma counter. The percent of the initial total radioactivity (in cpm) was then calculated at each time point. The $\log_{10}$ of the % total cpm was then plotted against time. The half-lives of peptide apoB602 in both wildtype and transgenic mice was then calculated from the slope of the decay line.

***In vivo* effect on lipoprotein(a) formation**

Human apo(a) transgenic mice were bred to human apoB transgenic mice to generate double transgenic mice containing lipoprotein(a) in the plasma. Lipoprotein(a) levels were measured using a sandwich ELISA and ranged from 12 to 32 mg per dl. Pairs of mice were matched for lipoprotein(a) levels. One pair was administered 200 µl of saline via tail vein injection, another pair 200 µl of a 10 mg/ml solution of the 602 peptide in saline (total dose = 2mg), and another pair a similar dose of the arg602 peptide.in saline. Blood samples were taken at 0, 0.17, 0.5 1, 2, 4, and 8 hrs after injection and lipoprotein(a) levels determined by a sandwich ELISA. In addition the levels of lipoprotein(a) versus free apo(a) were visualised by Western blot analysis.

RESULTS/DISCUSSION

***In vivo* properties of the apoB peptides**

Evaluation of peptide half-life in the circulation of wild type mice (Figure 5) revealed a half-life of 55 minutes for the apoB602 peptide. Interestingly, the half life of the apoB602 peptide was increased threefold in the circulation of apo(a) transgenic mice (t1/2 = 3 hours) suggesting that the apoB602, peptide was interacting with apo(a) and hence its clearance from plasma was being retarded.

A pilot study was performed to test the effect of the apoB602 and arg602 peptide in lipoprotein(a) mice. While there was no statistically significant effect on lipoprotein(a) levels in mice administered either the apoB602 or arg602 peptide (data not shown), there was an increase in the amount of free apo(a) levels in mice treated with the arg602 peptide (Figure 6) suggesting that the assembly of lipoprotein(a) was being disrupting by the arg602 peptide.

INDUSTRIAL APPLICATION

Thus, in accordance with the present invention, there are provided proteins (antibodies and a family of peptides) having the capability of at least partially inhibiting the formation of lipoprotein(a). This inhibitory function means that the peptides and antibodies of the invention and the medicaments containing them have utility as lipoprotein(a) lowering agents. In turn, this has important implications in the strategy for preventing or treating diseases such as atherosclerosis.

It will be appreciated by those persons skilled in the art that the above description is provided by way of example only and that modifications are contemplated in terms of different amino acid sequences and different peptide lengths as part of the invention.

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CLAIMS

1. A peptide which at least partially inhibits lipoprotein(a) formation which comprises the following amino acid sequence:

$$R_1 X X X X X X R_2 X X X X X X R_3 X X X X X X R_4$$

wherein R_1, R_2, R_3 and R_4 are each independently selected from lysine, arginine and histidine, and wherein each X is an amino acid other than lysine, arginine or histidine.

2. A peptide according to claim 1 wherein R_1, R_2, R_3 and R_4 are all lysine.

3. A peptide according to claim 1 wherein at least three of R_1, R_2, R_3 and R_4 are lysine.

4. A peptide according to claim 3 wherein three of R_1, R_2, R_3 and R_4 are lysine and the other is arginine.

5. A peptide according to claim 1 wherein R_1, R_2, R_3 and R_4 are all arginine.

6. A peptide according to claim 1 wherein at least three of R_1, R_2, R_3 and R_4 are arginine.

7. A peptide according to claim 6 wherein three of R_1, R_2, R_3 and R_4 are arginine and the other is lysine.

8. A peptide according to claim 1 which comprises the following amino acid sequence:

K Y Y E L E E K I V S L I K N L L V A L K.

9. A peptide according to claim 8 which has the amino acid sequence:

K Y Y E L E E K I V S L I K N L L V A L K.

10. A peptide according to claim 8 which has the amino acid sequence:

S I V G W T V K Y Y E L E E K I V S L I K N L L V A L K D F H S.

11. A peptide according to claim 5 which comprises the following amino acid sequence:

R Y Y E L E E R I V S L I R N L L V A L R.

12. A peptide according to claim 11 which has the amino acid sequence:

R Y Y E L E E R I V S L I R N L L V A L R.

13. A peptide which at least partially inhibits lipoprotein(a) formation which comprises the following amino acid sequence:

K Y Y E L E E K I V S L I K N L L V A L K

or a functional equivalent thereof.

14. A peptide which has an alpha-helical structure and which comprises the following amino acid sequence:

K X X X X X X K X X X X X K X X X X X X K

wherein X is any amino acid other than lysine, and wherein said peptide is capable of at least partially inhibiting lipoprotein(a) formation.

15. A peptide which is at least 21 amino acid residues in length, which forms an alpha-helical structure with four surface residues independently selected from lysine, arginine or histidine and which is capable of at least partially inhibiting lipoprotein(a) formation.

16. A peptide according to claim 14 which includes four surface lysine residues.

17. A peptide according to claim 14 which includes four surface arginine residues.

18. A polynucleotide which encodes a peptide as claimed in any one of claims 1 to 17.

19. A polynucleotide which encodes a peptide as claimed in claim 8 or claim 9.

20. A polynucleotide according to claim 19 which has the sequence:

AAA TAT TAT GAA CTT GAA GAA AAG ATA GTC AGT CTG ATC
AAG AAC CTG TTA GTT GCT CTT AAG.

21. A polynucleotide which encodes a peptide as claimed in claim 10.

22. A polynucleotide which encodes a peptide as claimed in claim 11 or claim 12.

23. An antibody which binds a peptide as claimed in any one of claims 1 to 17 and which is capable of at least partially inhibiting lipoprotein(a) formation.

24. An antibody which binds to apoB region apoB 4372-4392.

25. An anti-idiotypic antibody which mimics the conformation of apoB region apoB 4372-4392 and which is capable of non-covalently binding apo(a).

26. A medicament which comprises a peptide according to any one of claims 1 to 17 or an antibody according to any one of claims 23 to 25 in a pharmaceutically acceptable form, said peptide being present in an amount sufficient to inhibit lipoprotein(a) formation.

27. The use of a peptide according to any one of claims 1 to 17 or an antibody according to any one of claims 23 to 25 in the manufacture of a lipoprotein(a)-lowering medicament.

28. A method of lowering lipoprotein(a) levels in plasma in a patient comprising the step of administering to said patient an amount of a peptide according to any one of claims 1 to 17, an antibody according to any one of claims 23 to 25, or a medicament according to claim 26 which is effective to at least partially inhibit new lipoprotein(a) formation.

29. A method of lowering lipoprotein(a) levels in a patient comprising the step of preventing or reducing effective non-covalent binding between apoB region apoB 4372-4392 and apo(a).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(1) APPLICANT: UNIVERSITY OF OTAGO

(2) TITLE: INHIBITION OF LIPOPROTEIN FORMATION

(3) NUMBER OF SEQUENCES: 4

(5) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5"HD FLOPPY DISC

(B) COMPUTER: IBM PC COMPATIBLE

(C) OPERATING SYSTEM: MS-DOS

(D) SOFTWARE: WORD 97

(2) INFORMATION FOR SEQUENCE ID NO. 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 1

Lys Tyr Tyr Glu Leu Glu Glu Lys Ile Val Ser Leu 12

Ile Lys Asn Leu Leu Val Ala Leu Lys 21

(2) INFORMATION FOR SEQUENCE ID NO. 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 2

Ser Ile Val Gly Trp Thr Val Lys Tyr Tyr Glu Leu 12

Glu Glu Lys Ile Val Ser Leu Ile Lys Asn Leu Leu 24

Val Ala Leu Lys Asp Phe His Ser 32

(2) INFORMATION FOR SEQUENCE ID NO. 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 3

Arg Tyr Tyr Glu Leu Glu Glu Arg Ile Val Ser Leu 12

Ile Arg Asn Leu Leu Val Ala Leu Arg 21

(2) INFORMATION FOR SEQUENCE ID NO. 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 63 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 4

AAATATTATG AACTTGAAGA AAAGATAGTC AGTCTGATCA 40

AGAACCTGTT AGTTGCTCTT AAG 63

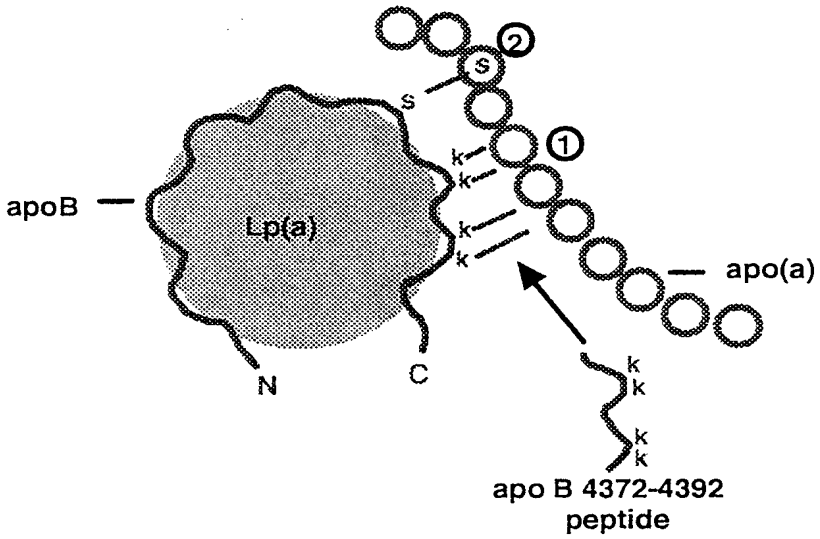


FIGURE 1

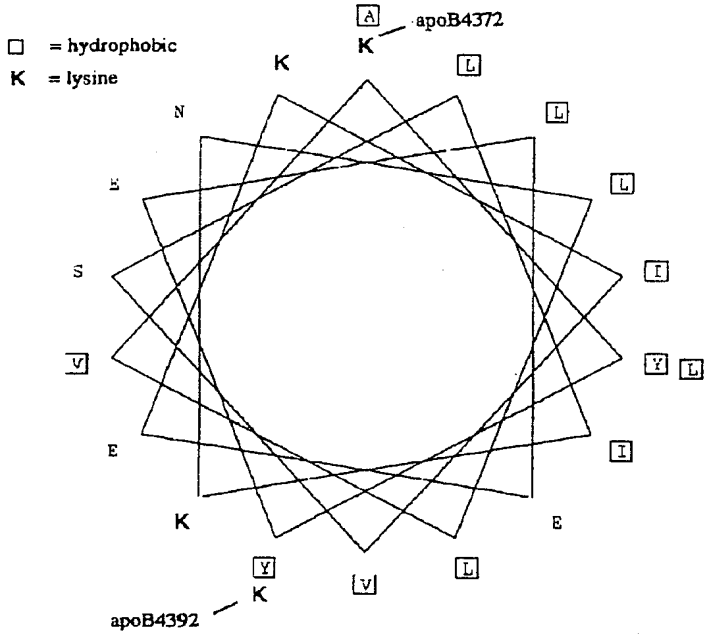

□ = hydrophobic
K = lysine
A
K
apoB4372
K
L
L
L
I
Y
L
I
E
L
V
K
apoB4392
Y
K
E
S
N
E

FIGURE 2

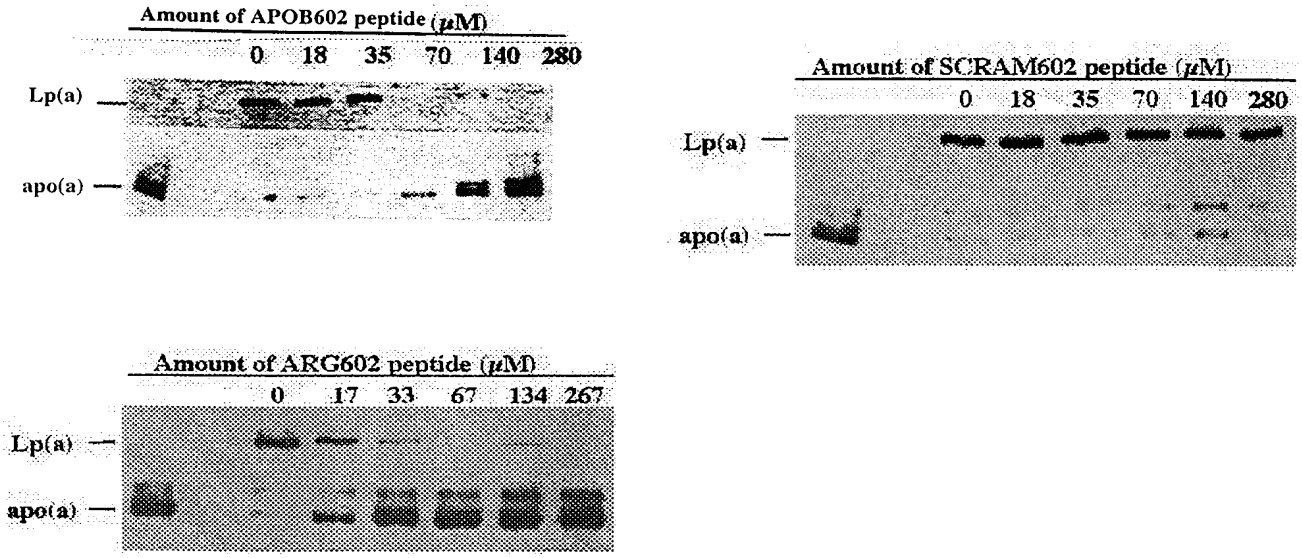

Amount of APOB602 peptide (μM)
0 18 35 70 140 280
Lp(a)
apo(a)
Amount of SCRAM602 peptide (μM)
0 18 35 70 140 280
Lp(a)
apo(a)
Amount of ARG602 peptide (μM)
0 17 33 67 134 267
Lp(a)
apo(a)

FIGURE 3

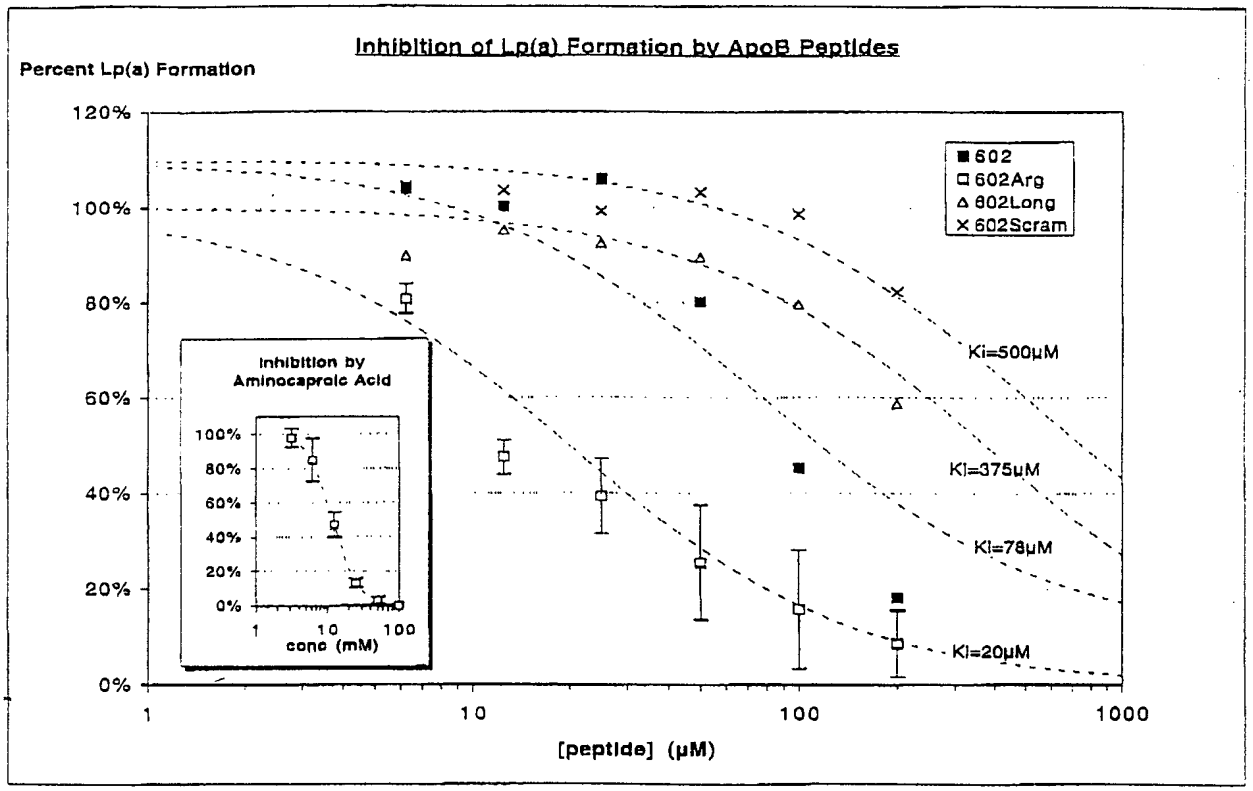

Inhibition of Lp(a) Formation by ApoB Peptides
Percent Lp(a) Formation
120%
100%
80%
60%
40%
20%
0%
1
10
100
1000
[peptide] (μM)
602
602Arg
602Long
602Scram
Ki=500μM
Ki=375μM
Ki=78μM
Ki=20μM
Inhibition by Aminocaproic Acid
100%
80%
60%
40%
20%
0%
1
10
100
conc (mM)

FIGURE 4

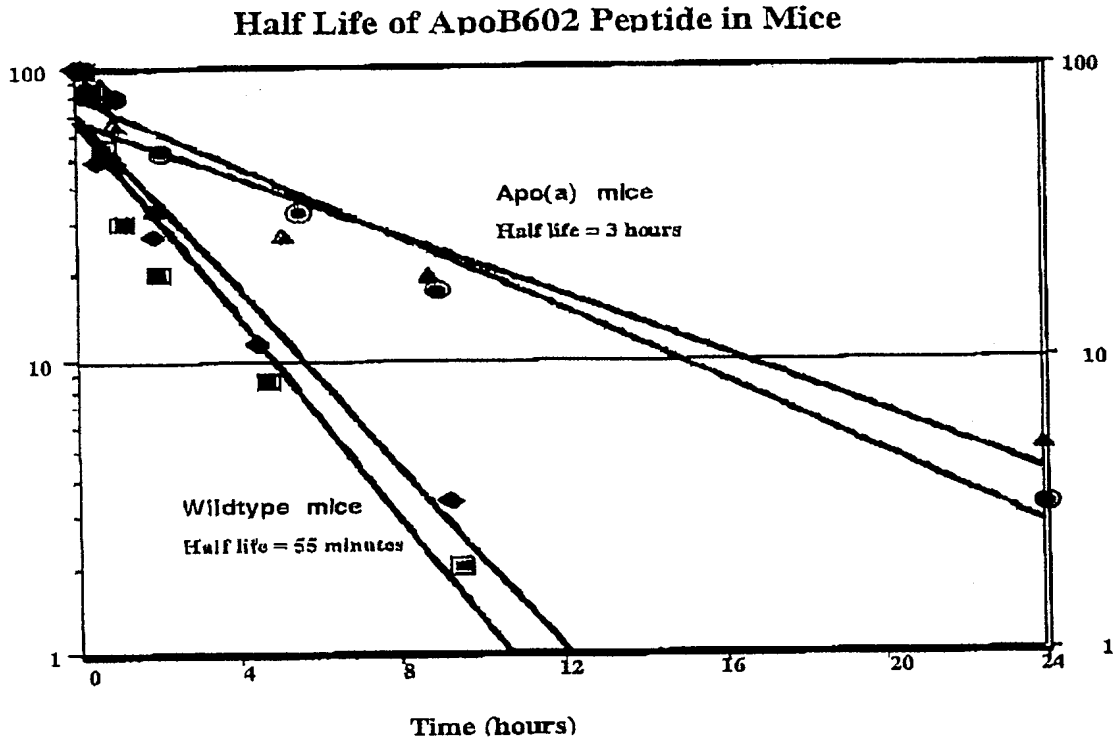
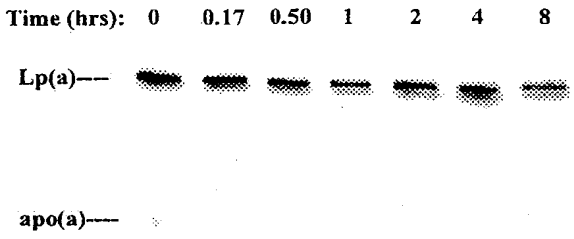
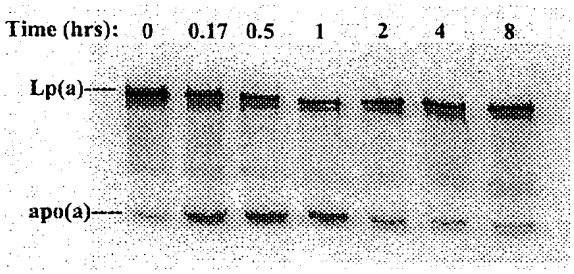

Half Life of ApoB602 Peptide in Mice
100
10
1
Apo(a) mice
Half life = 3 hours
Wildtype mice
Half life = 55 minutes
0
4
8
12
16
20
24
Time (hours)

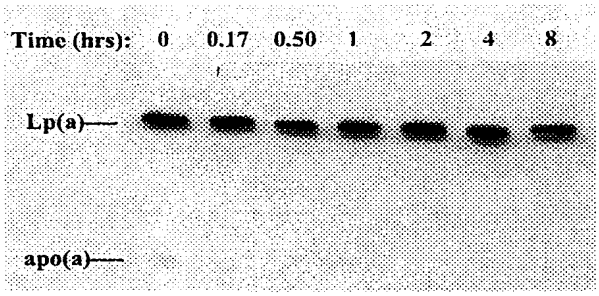
FIGURE 5

Saline Treated



Arg602 Treated





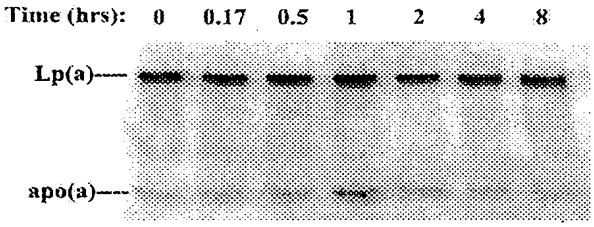


FIGURE 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ 99/00109

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C07K 14/775

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC AS ABOVE

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT: Sequence search via dgene ([KRH]YYELEE[KRH]IVSLI[KRH]NLLVAL[KRH])
ANGIS: Seq Id 1 & 3

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Science, v. 230, 1985, pp 37-43, KNOTT T.J. *et al*, "Human apolipoprotein B: structure of carboxyl-terminal domains, sites of gene expression, and chromosomal localization"	
A	J. Biol. Chem., v. 261, 1986, pp 12918-21, CHEN S.H. *et al*, "The complete cDNA and amino acid sequence of human apolipoprotein B-100"	
A	Gene, v. 70, 1988, pp 213-29, MAEDA N. *et al*, "Molecular genetics of the apolipoprotein B gene in pigs in relation to atherosclerosis"	

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 October 1999	14 OCT 1999
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929	Authorized officer **BARRY SPENCER** Telephone No.: (02) 6283 2284

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ 99/00109

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO, A, 97-43311 (Royal Free Hospital School Medicine) 20 November 1997	
P,A	WO, A, 98-56938 (Baylor College Medicine) 17 December 1998	

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/NZ 99/00109

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member			
WO	97/43311	EP	917539	GB	96/09702
WO	98/56938	AU	81401/98		

END OF ANNEX